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Distribution and genetic diversity of the invasive pest *Halyomorpha halys* (Hemiptera, Pentatomidae) in Belgium

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Abstract

The brown marmorated stink bug, *Halyomorpha halys*, native to East Asia, is an invasive pest of economic importance. It has invaded North America and many European countries and is further expanding its range. In Belgium, it was first observed in 2011. *Halyomorpha halys* is known to cause severe damage in commercial fruit orchards and vegetable crops. A dramatic and unmitigated expansion of *H. halys* in its adventive range could lead to significant economic implications for agricultural production. In this study, occurrence data of *H. halys* since its first observation in Belgium was analysed together with molecular information to map the populations and evaluate the genetic diversity of this pest. The genetic diversity of *H. halys* in Belgium was compared to data from other invaded and native countries reported in previous studies to identify possible invasion routes. The analysis of 1176-bp of mitochondrial DNA cytochrome c oxidase I and II genes (COI and COII) led to the discovery of two novel COI-COII haplotype combinations currently unique to Belgium. The invasion of *H. halys* in Belgium is likely the result of multiple and ongoing introductions from its native region and from already invaded countries in Europe, particularly Italy. The expansion of the brown marmorated stink bug populations in Belgium is recent and ongoing. Presently, it appears to thrive best in northern Belgium.

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Keywords
brown marmorated stink bug, haplotype diversity, Heteroptera, population genetics

Introduction

Halyomorpha halys (Hemiptera: Pentatomidae) or the brown marmorated stink bug is a stink bug of economic importance, native to East Asia and an invasive pest species in Europe, North America, and other regions (Hoebeke and Carter 2003; Leskey et al. 2012; Lee et al. 2013; Haye et al. 2015; Zhu et al. 2016; Hamilton et al. 2018). In Europe, *H. halys* has already been detected as far north as The Netherlands (Aukema et al. 2019), Belgium (Claerebout et al. 2018) and the north of Germany (Hartung et al. 2022). Due to its polyphagous nature, *H. halys* can feed on virtually all primary fruit and vegetable crops in its invaded areas (Kuhar et al. 2012; Haye et al. 2015). For example, in northern Italy, it has become a key pest in fruit orchards, with an estimated economic impact of €588 million on the production of pear, apple, peach, and kiwi in 2019 (Bulgarini et al. 2020). *Halyomorpha halys* causes damage through its feeding activity. It inserts its stylets into the plant and injects saliva, pre-digesting the plant tissue before feeding on it (Rice et al. 2014). This feeding behaviour causes scarring, deformities, pitting, and discolouration of the product, rendering it unmarketable or even inedible (Rice et al. 2014; Bulgarini et al. 2020). Since its first record in Belgium in 2011, observations of *H. halys* on public citizen science databases (Observation International and local partners 2022) only appeared in 2017. Currently, it is assumed that *H. halys* has established univoltine overwintering breeding populations in Belgium (Claerebout et al. 2018). An observation of *H. halys* from the Haspengouw region in July 2021 indicated that *H. halys* is already present in commercial pip fruit orchards in Belgium. However, to date, no dramatic population increases, mass occurrences or damage has been reported in fruit production in Belgium (Berteloot et al. 2021). In 2022, in Belgium, 219.000 t of apples and 366.000 t of pears, some of the most vulnerable fruits to *H. halys*, were produced (European Commission 2022a, b). Apples and pears represent a combined revenue of more than € 148 million of which pear production is the most significant part with a revenue of € 119 million (Verbond van Belgische Tuinbouwcoöperaties 2022). An unmitigated expansion of *H. halys* in Belgium could therefore have a considerable impact on fruit production, especially in the Flanders region, which produces most of the apples, pears, and soft fruits in the country.

The increased accessibility and affordability of molecular technologies, as well as the expansion of databases containing publicly available DNA sequence data, have aided in the use of molecular tools to assess the genetic diversity and potential origin of invasive species (Cristescu 2016; Hamelin and Roe 2019). Due to its lack of recombination, relative neutrality and shorter coalescence time, mitochondrial DNA (mtDNA) is a highly popular molecular marker for examining genetic diversity and phylogeography of (invasive) animal species (Ficetola et al. 2008; Rollins et al. 2011;

Bras et al. 2019; Ryan et al. 2019). Furthermore, for invasive species, the accumulation of easily reproducible genetic data in public databases facilitates the regular addition of new data from the invasion front to build a global pattern of invasion dynamics. For example, for *H. halys*, mtDNA sequences (portions of the mitochondrial gene cytochrome c oxidase subunits I and II – COI and COII, respectively) are already available for both native (China, Japan, Korea) and invaded countries (Gariepy et al. 2014; Xu et al. 2014; Cesari et al. 2015; Cesari et al. 2018; Lee et al. 2018; Yan et al. 2021).

In this study, we set out to fill one of the geographical knowledge gaps in Europe on the genetic diversity and distribution of *H. halys*. First, we investigated the genetic diversity of *H. halys* in Belgium by sequencing 99 specimens from 18 locations at two mitochondrial genes: COI and COII. Then, we compared those sequences to previously published *H. halys* sequences to infer the putative invasion routes to Belgium. Lastly, we used public citizen occurrence data to map the distribution of *H. halys* in Belgium from 2020 to 2022 and gain demographic insights into the Belgian populations.

Methods

Insect sampling

Halyomorpha halys specimens were collected from 18 locations in Belgium (Suppl. material 1) from agricultural sites and private gardens in 2021 and 2022 using baited traps (dual lure from Trécé Inc., Adair Oklahoma, USA) or by beating of the lower part of a tree trunk/shrub. The collected samples were stored dry or in 70% ethanol at -20 °C until used for molecular analysis.

DNA extraction & sequencing

DNA was extracted from two legs using a Chelex extraction method (Walsh et al. 1991). Briefly, the legs were crushed with a sterile pestle in 100 µl of 5% Chelex 100 (Bio-Rad, USA) solution. They were then incubated at 85 °C for 90 min, and the supernatant was collected after 3 min of centrifugation at 12,000 rpm. The cytochrome oxidase subunit I (COI) and II (COII) barcode regions of the mitochondrial DNA were amplified through PCR. For the COI region, the LCO 1490 (5'- GCTCAACAAATCATAAAGATATTGG-3') and HCO 2198 (5'- TAAACTTCAGGGTGACCAAAAAATCA-3') primers (Folmer et al. 1994) were used. For COII, the HhalysCO2F2 (5'-TAACCCAAGATGCAAATTCT-3') and HhalysCO2R2 (5'- CCATATATAATTCCTGGACGA-3') primers (Xu et al. 2014) were used. For both regions, the following PCR cycles were used: initial denaturation at 94 °C for 3 min, 38 cycles of denaturation at 94 °C for 30 sec, annealing at 48 °C for 30 sec and extension at 72 °C for 45 sec, followed by a final extension step at 72 °C for 7 min. The PCR product quality was checked on agarose gel. Both strands (forward and reverse) for each barcode region were sequenced (Eurofins, Germany GmbH) by Sanger sequencing.

Haplotype diversity analysis

Forward and reverse sequences were trimmed and assembled into a consensus sequence using CodonCode Aligner (version 10.0.2). COI and COII sequences obtained in this study were compared to COI and COII sequences from Yan et al. (2021) and Cesari et al. (2018), retrieved from GenBank and BOLD. Other previously conducted studies on the genetic diversity of *H. halys* only sequenced COI or COII fragments (or only reported unique haplotypes found without specifying which samples are associated with which haplotypes) (Gariepy et al. 2014; Xu et al. 2014; Valentin et al. 2017; Lee et al. 2018; Kapantaidaki et al. 2019). All individual COI and COII haplotypes found in this study have already been reported in other publications (see results) and were given the haplotype name used in those publications (Cesari et al. 2018; Yan et al. 2021). However, for COI, there were discrepancies between the sequences of haplotypes with the same name found in Cesari et al. (2018) and Yan et al. (2021). Haplotypes found in our study were named after the reference haplotypes of the study of Valentin et al. (2017) if a sequence match was found. Haplotype names from Cesari et al. (2018) were distinguished with the letter "c" (Table 1). The sequence of haplotype H41 from Yan et al. (2021) perfectly matched the H41 haplotype from Valentin et al. (2017) but corresponded to the sequence of H46 from Cesari et al. (2018). Therefore, the name of the sequence of this haplotype was retained in the present study as H41. Another sequence found in this study without a match in Valentin et al. (2017) or Yan et al. (2021) was already named H41 in Cesari et al. (2018) and was renamed "H41c" to distinguish it. For H40 and H43, the same haplotype names were given to different sequences by Cesari et al. (2018) and Yan et al. (2021). Again, those sequences were compared to the ones of Valentin et al. (2017). The H40 and H43 sequences from Yan et al. (2021) perfectly matched the identical name sequences from Valentin et al. (2017), and these names were retained in the present study. A letter "c" was added to the H40 and H43 sequences from Cesari et al. (2018) to distinguish them. Finally, the H42 and H49 sequences from Cesari et al. (2018) did not match the H42 and H49 haplotypes from Valentin et al. (2017) (no H42 and H49 haplotypes in Yan et al. (2021) for comparison). To be consistent, the letter "c" was added to the H42 and H49 sequences from Cesari et al. (2018).

Table 1. Summary of the discrepancies between sequences of COI-haplotypes with the same name in different studies and the names used in the present study.

Valentin et al. (2017)	Cesari et al. (2018)	Yan et al. (2021)	This study
H40	/	H40	H40
/	H40	/	H40c
H41	H46	H41	H41
/	H41	/	H41c
/	H42	/	H42c
H43	/	H43	H43
/	H43	/	H43c
/	H49	/	H49c

Sequences were aligned and analysed in R v4.0.2 (R Core Team 2020), using packages msa (Bodenhofer et al. 2015), adegenet (Jombart 2008) and pegas (Paradis 2010). The relationships among haplotypes within Belgium and between haplotypes from Belgium and other countries were investigated through haplotype networks and genetic diversity indexes (haplotype, Hd, and nucleotide diversity, π). We tested for the presence of a genetic structure in Belgium by performing a Mantel test (Euclidean) on genetic and geographic distance matrices between collected specimens.

Occurrence data

Occurrences from January 1st, 2017, to December 31st, 2022 were obtained from the publicly available citizen science database (Observation International and local partners 2022). For *H. halys*, the recorded occurrences on the public citizen science database are quality-controlled, and the taxonomic accuracy of each observation is confirmed or rejected by a specialist through photos. The occurrence data was additionally examined to check the validity of all the occurrences. Only occurrences with a high confidence level in the taxonomic identification of *H. halys* and with spatial coordinates were used. Additionally, specimens collected during this research were identified through morphological identification using the *H. halys* identification key from Maistrello et al. (2016) and confirmed by our molecular analysis. The occurrence data was visualised with QGIS 3.30.1 (QGIS Development Team 2023).

Results

DNA sequences

- COI: 99 *H. halys* sequences were retrieved out of 99 specimens collected. All specimens yielded a 658-bp DNA sequence. The DNA sequence data and specimen collection information were made accessible on GenBank with accession numbers OR581617–OR581715.
- COII: 93 sequences with a length of 518-bp were retrieved from 99 *H. halys* specimens collected. The DNA sequence data and specimen collection information were made accessible on GenBank with accession numbers OR602454–OR602546.

Haplotype diversity of *H. halys*

For the COI fragment individually, nine distinct haplotypes were found, consisting of 14 polymorphic sites (Fig. 1a). Three haplotypes were dominant (H01, H03 and H08) and were shared by roughly 80% of the individuals collected. Haplotype diversity was 0.77 ± 0.02 (mean ± SD). The obtained COI sequences from this study were compared with 609 COI sequences from other European countries, Chile, the USA, China, and Japan. H01 and H03 haplotypes are shared with those in invaded areas in

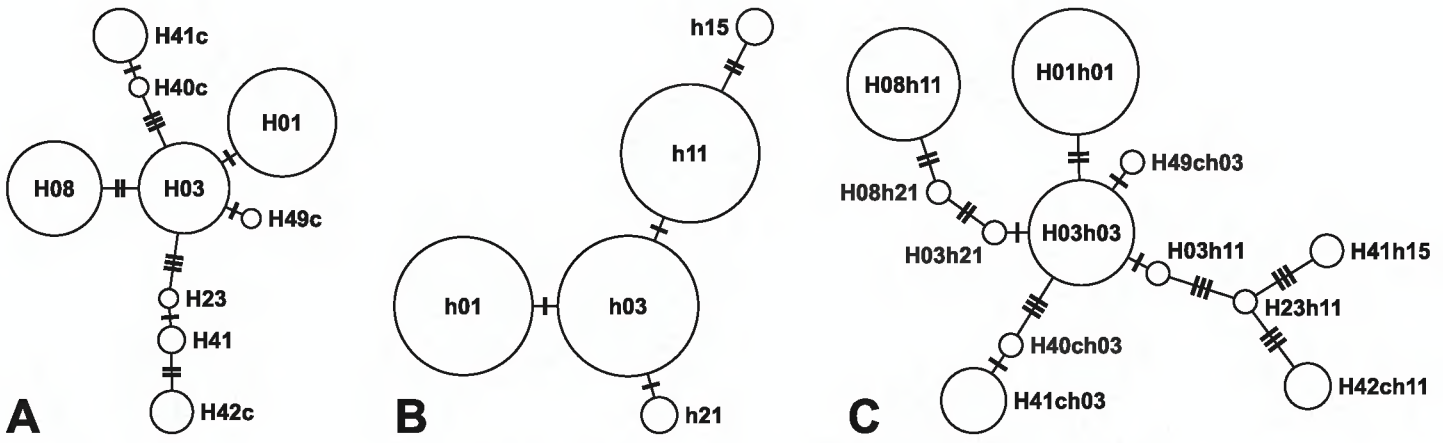

Figure 1. Haplotype network using **A** COI **B** COII and **C** COI-COII fragments of *H. halys* in Belgium. The circle size is proportional to the square root of the frequency of the haplotypes. The tick marks on each line represent a base pair difference.

Europe and America, as well as with those in the native area of China, but not with Japan. Haplotype H08 is shared only with some invaded areas in Europe (Austria and Italy). The remaining haplotypes are shared with Italy (H40c, H41, H41c, H42c and H49c), Japan (H23 and H41) and Hungary (H41).

For the COII fragment individually, five distinct haplotypes were found, consisting of 5 polymorphic sites (Fig. 1b). Haplotype diversity was 0.70 ± 0.01. Our COII sequences were compared with 651 COII sequences from other European countries, Chile, the USA, China, and Japan. Haplotypes h01, h03 and h11 accounted for 91 out of 95 haplotypes from Belgium. Haplotypes h01 and h03 are shared with invaded areas in Europe, the USA and China but not with Japan. Haplotypes h11 and h15 are only shared with some other European countries (Austria, Hungary, and Italy) and Japan. Haplotype h21 is shared only with Italy.

The resulting concatenated 1176-bp sequences rendered 12 distinct COI-COII haplotypes among 95 specimens consisting of 22 polymorphic sites in total (Fig.1c). Two novel COI-COII haplotypes were identified (H03h11 and H08h21) in our samples; these haplotypes are unique to Belgium. The ten other haplotype combinations were previously reported in the literature. The comparison of COI-COII haplotypes from Belgium to previously published sequences confirm the pattern observed for individual loci. The most frequent haplotype H01h01 is shared with all native and invaded countries included in this study (Table 2). H03h03, the second most frequent haplotype, has been recorded in Austria, Chile, China, Italy, and Hungary. Some of the remaining haplotypes (H03h11, H03h21, H41ch03 and H49ch03) are only shared with Italy (and Austria for H08h11) and are close to haplotypes from China. Others (H23h11, H41h15 and H41ch11) are shared with Italy (and Hungary for H41h15) and/or with (or close to) Japan (H46h15, H42h11, H23h11 and H08h11). Finally, the H08h21 and the H03h11 haplotypes are unique to Belgium and are close to the haplotypes from Italy (within the group of haplotypes found in most invaded countries and China).

Table 2. Summary table of mtDNA (COI-COII) diversity by country. With N: sample size, Hn: number of haplotypes, h: haplotype diversity and π: nucleotide diversity (only countries with available COI-COII sequences are listed).

Continent	Country	First record	N	Hn	Hd ± SD	π ± SD	Study
Asia	China	Native	90	24	0.86 ± 0.02	0.0033 ± 0.0018	Yan et al. 2021
	Japan	Native	65	32	0.94 ± 0.01	0.0024 ± 0.0014	Yan et al. 2021
	Turkey	2017 (Güncan and Gümüş 2019)	11	1	0	0	Yan et al. 2021
Europe	Austria	2015 (Rabitsch and Friebe 2015)	15	4	0.69 ± 0.10	0.0021 ± 0.0013	Yan et al. 2021
	Belgium	2011 (Claerebout et al. 2018)	95	12	0.79 ± 0.02	0.0031 ± 0.0018	This study
	Georgia	2015 (Gapon 2016)	31	1	0	0	Yan et al. 2021
	Greece	2011 (Milonas and Partsinevelos 2014)	8	3	0.61 ± 0.16	0.0025 ± 0.0017	Cesari et al. 2018
	Hungary	2014 (Vétek et al. 2014)	90	3	0.11 ± 0.04	0.0003 ± 0.0003	Yan et al. 2021
	Italy	2012 (Maistrello et al. 2014)	16	18	0.72 ± 0.03	0.0028 ± 0.0016	Cesari et al. 2018; Yan et al. 2021
	Romania	2015 (Macavei et al. 2015)	30	1	0	0	Cesari et al. 2018
	Serbia	2015 (Šeat 2015)	13	5	0.61 ± 0.07	0.0014 ± 0.0008	Yan et al. 2021
	Slovenia	2017 (Rot et al. 2018)	15	3	0.51 ± 0.12	0.0012 ± 0.0008	Yan et al. 2021
North America	United States	2001 (Hoebeke and Carter 2003)	24	1	0	0	Yan et al. 2021
South America	Chile	2017 (Faúndez and Rider 2017)	31	2	0.06 ± 0.06	0.0001 ± 0.0002	Yan et al. 2021

The results of our Mantel test indicated a significant but weak correlation ($r = 0.14$, $p = 0.001$) between the genetic distance and the geographical distances for pairs of individuals. High haplotype diversity was observed in Belgium, Hd = 0.79 ± 0.02, with a nucleotide diversity value of $\pi = 0.0031 \pm 0.0018$ (Table 2). This level of genetic diversity was lower than that of native regions Japan and China (Hd = 0.94 ± 0.014 and 0.86 ± 0.023, respectively) but similar to Italy (Hd = 0.72 ± 0.033) (Table 2). Other invaded areas of *H. halys* typically had lower haplotype diversity but their estimation may be approximate due to shallower sampling depth (low sample size and/or few sampling locations; Table 2). The genetic data analysis of this research is publicly available through the following link: https://zenodo.org/records/10210286.

Distribution of *H. halys* in Belgium

The 740 observations from 6 years (2017–2022) were checked for accuracy and completeness. Since the first record in 2011, occurrences in subsequent years initially remained low, without any public citizen database records or specimens collected until 2017. *Halyomorpha halys* was recorded once in 2017 and 2018, 5 times in 2019 and 35 times in 2020. In recent years, the number of observations of *H. halys* has increased dramatically. In 2021, 183 observations were recorded, followed by a substantial increase to 515 in 2022. Up until November 2023, the Belgian public citizen database reported more than 2200 observations, a more than tenfold increase compared to 2021. From the occurrences of *H. halys* in 2020–2022, most of the observations were made in northern Belgium, mainly around the urban areas of the cities of Gent, Leuven, and Mechelen and the region of Haspengouw (Fig. 2a–c).

Figure 2. Map of Belgium with recorded occurrences of *H. halys* in **A** 2020 **B** 2021, and **C** 2022. Each dot is an individual record, and density clouds indicate the level of density of occurrences in one area.

Discussion

This study uncovered 9 COI, 5 COII and 12 COI-COII distinct haplotypes from 99 *H. halys* specimens collected in Belgium. Among these, two new COI-COII haplotype combinations (H03h11 and H08h21), currently unique to Belgium, were observed.

The principal COI haplotypes present in Belgium were H01 (33%), H08 (24%) and H03 (23%). H01 and H03 are the most frequent haplotypes in China and in most invaded countries (Gariepy et al. 2014; Valentin et al. 2017; Cesari et al. 2018; Lee et al. 2018; Yan et al. 2021; Gariepy et al. 2021). As such, their presence in Belgium is to be expected and therefore does not help to determine the origin of *H. halys* in Belgium. Interestingly, however, H08 is the second most abundant haplotype in Belgium. So far, this haplotype has been exclusively detected in certain European countries: Austria, France, Italy, Switzerland and Serbia (Valentin et al. 2017; Cesari et al. 2018; Gariepy et al. 2021; Yan et al. 2021). This suggests that at least part of the *H. halys* population in Belgium originates from previously invaded territories in Europe (i.e., bridgehead effect; Lombaert et al. 2010). Remarkably, eight out of the nine COI haplotypes found in Belgium are shared with Italy. Furthermore, half of these haplotypes were reported exclusively in these two countries (H40c, H41c, H42c and H49c) suggesting that Italy is a direct region of origin of *H. halys* in Belgium. The rare H23 haplotype was only found once in Belgium and has been reported in Japan and the Western USA (Oregon) (Valentin et al. 2017). It was also detected in a shipment in Australia originating from Italy, suggesting that the H23 haplotype is also present in Italy (Horwood et al. 2019; Gariepy et al. 2021). Introductions may thus have originated from Japan or the USA in addition to Italy.

For the COII fragment, h01, h03 and h11 each accounted for 32% of the abundance. The h01 and h03 haplotypes are native to China and Korea (but have not been reported in Japan yet) and are the most frequent COII haplotypes in invaded countries (Xu et al. 2014; Cesari et al. 2018; Yan et al. 2021). The distribution of the h11 COII haplotype is more restricted and has only been reported in Austria, Italy, Japan, and Korea (Xu et al. 2014; Cesari et al. 2018; Yan et al. 2021). Consistent with Italy as a source of *H. halys* for Belgium, the h21 haplotype was isolated from two specimens collected in Belgium and is only shared with Italy (Cesari et al. 2018).

In this study, the combination of COI and COII fragments did not result in a significantly better geographic resolution to reveal possible origins of the invasion.

However, the presence of haplotype H03h21 provides some additional support for Italy as a source of the invasion since this combination is only shared with a sample from the Piedmont region of Italy (Cesari et al. 2018). Additionally, the inclusion of the COII fragment in this study revealed two novel haplotype combinations unique to Belgium: H08h21 and H03h11. The former is likely to originate from Italy since haplotype h21 has been exclusively reported there (Piedmont region, same sample as for H03h21), while H08 has been detected multiple times (Piedmont and Lombardy regions of Italy). H03h11 is more singular because it combines a COI haplotype native to China (H03) and a COII haplotype native to Korea or Japan (h11; see above). Such a combination could arise from recent COI or COII mutations. H03 is only two substitutions from the closest COI haplotype native to Japan (H27), and h11 is only one substitution from three COII haplotypes native to China (h03, h05 and h10). Alternatively, this combination could also exist in the regions of origin (China and/or Japan/Korea) but has not yet been sampled. Belgium's dominant haplotypes generally overlap with those from previously invaded European countries, such as Italy, Switzerland, Austria, and Hungary. In contrast, rarer haplotypes are exclusively shared with Italy and its native region of Japan (H23).

The genetic diversity found in Belgium is surprisingly high (Hd = 0.79, π = 0.0018, N = 95) compared to other invaded countries previously studied (Table 2), several distinct haplotypes were isolated from single sampling sites (Suppl. material 1). A reduced genetic diversity is usually expected for introduced species because of a limited number of founders associated with early genetic drift. However, genetic bottlenecks associated with introductions can be counteracted if many individuals are introduced at the same time or if repeated introductions occur (Dlugosch and Parker 2008). Similarly, Schuler et al. (2020) found a high haplotype diversity (Hd = 0.68, π = 0.0046, N = 156) in the *H. halys* population of South Tyrol in Northern Italy. The population in Tyrol was established by a secondary introduction from Eastern USA into the Emilia Romagna region of Italy and the spread of the founder population in Switzerland to the neighbouring countries. In contrast, Valentin et al. (2017) concluded that most invasive populations in North America and Europe were established from a direct introduction of *H. halys* from China with separate introductions into Eastern and Western USA and Canada, as well as into Switzerland and Greece. The high genetic diversity of *H. halys* populations in Belgium and the overlap of haplotypes with previously invaded European countries, such as Italy, Switzerland, Austria, and Hungary but also with Japan indicates that the Belgian populations probably originated from multiple invasions from already invaded European countries, mainly Italy, but also directly from Japan through inadvertent human-mediated transportation (often due to global trade of goods) (Valentin et al. 2017). The weak genetic structure detected by our Mantel test could be related to the recent local expansion of these genetically diverse clusters.

Previous studies have shown that citizen science provides valuable data to characterise the spread of *H. halys* (Maistrello et al. 2016; Stoeckli et al. 2020; Streito et al. 2021). From our data in Belgium, more *H. halys* observations are recorded in the nosrthern part of Belgium compared to the southern part of Belgium, with more urban

areas than agricultural, forested, and rural areas. However, on a national scale, spatial biases are commonly associated with human population density, settlements, and road infrastructure (Kelling et al. 2015; Geldmann et al. 2016; Girardello et al. 2019. The bias towards urban areas in citizen-collected occurrence data and rising popularity of tools to make observations (e.g., smartphones) represents a challenge to infer true spatio-temporal patterns from our occurrence data (Bowler et al. 2022). Nevertheless, it also provides natural experimental gradients to examine the impacts of future environmental scenarios including climate change (Lahr et al. 2018). Urban areas are often seen as heat islands, offering more suitable refugia to survive winters or more favourable conditions throughout the seasons to develop and reproduce. Therefore, these urbanised areas often comprise a larger population of insects (Kaiser et al. 2016; Frank and Backe 2023). According to Kistner (2017), the northern part of Belgium lies near the latitudinal border of the climate in Europe suitable for the winter survival and summer reproduction of *H. halys*. In contrast, the southern part of Belgium is currently modelled to be an unsuitable eco-climatic region for *H. halys*, with colder summer and winter temperatures and fewer refugia to survive winter due to the lower urbanisation (Kistner 2017). Niche modelling through climate suitability by Streito et al. (2021) essentially confirms this, with the northern part of Belgium having more suitable areas for *H. halys* while the southern part is more marginal to unsuitable for its survival and expansion. Despite the possibility of spatio-temporal bias in our occurrence data, some temporal trends can still be derived from citizen-collected occurrence data (Powney et al. 2019; Outhwaite et al. 2020; Sheard et al. 2021; Zattara and Aizen 2021; Bowler et al. 2022). Our observational data shows an almost fifteenfold increase in observations from 2020 to 2022. However, to date, no damage related to *H. halys* has been reported in agricultural production in Belgium (Berteloot et al. 2021). Based on the occurrence data, it is likely that the population expansion of *H. halys* in Belgium is very recent, as a relatively high number of observations were only made in 2021, 2022 and 2023. Streito et al. (2021) define three main phases in the invasion of *H. halys*: (1) the very beginning of the invasion, when populations of *H. halys* are low, and naturalists and official monitoring services who are excellent observers and expecting the emergence of *H. halys* can detect its presence, (2) when abundance increases, non-naturalist citizens are able to detect the species and start to provide information on the dynamics of the invasion and indirectly on the level of populations through citizen science platforms, and (3) when populations have expanded and become large enough, agricultural professionals take over and can predict and assess the population density and damages. From our occurrence data, the *H. halys* population in Belgium is seemingly under expansion, being numerously recorded in the citizen science databases.

Lastly, the univoltine *H. halys* population in Belgium likely expands in the summer and declines in the winter in current climatic conditions due to Belgium being situated at the northern latitudinal border of climatic suitability for *H. halys*, with winters cold enough to kill more individuals than winter temperatures in southern European countries like Italy, possibly delaying the fast expansion of its populations. However, both parts of Belgium are modelled to be suitable for the survival and development of *H. halys* by 2100 (Kistner 2017).

Conclusions

The results of this study provide haplotype information for *H. halys* from a newly invaded region. The haplotype diversity in Belgium is surprisingly high, with 9 COI, 5 COII and 12 COI-COII haplotypes found. The invasion of *H. halys* in Belgium likely occurred repeatedly and is assumed to be still ongoing through human-mediated transportation from other invaded European countries and directly from its native regions in Eastern Asia. A significant overlap between Belgian and Italian haplotypes points to Italy as the most probable source for a significant proportion of haplotypes currently present in Belgium. By combining the citizen-collected occurrence data with the molecular data, we assume the population expansion of *H. halys* is recent and ongoing.

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Supplementary material 1

Metadata of all samples

Authors: Olivier Hendrik Berteloot, Alexandre Kuhn

Data type: csv

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